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Protocols for FACS purification of cardiac total interstitial single cells from adult mouse cardiac ventricles

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An abbreviated version of this protocol was published in eLIFE in Mar 2019

Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury

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Detailed protocol

Before you start:

- Set all centrifuges to 4 ^{o}C
- Set the water bath to 37 ^{o}C and adjust the water level, enough to cover half of the specimen jar.
- Place the collagenase type Ⅱ stock bottle in a desiccator at room temperate (RT) for at least 30 mins before opening.
- Pre-warm the red cell lysis buffer to RT.

Reagents to prepare:

- **FACS buffer**: Prepare 2% FBS in PBS and filter through 0.2 µm syringe filter.
- **Collagenase type Ⅱ in PBS (working concentration: 265 U/ml):** Prepare fresh solution each time. Dissolve collagenase in PBS and filter through 0.2 µm syringe filter. Note: check the dry concentration (U/mg) of the protein as specified by the manufacturer and prepare normalized working solution based on the unit concentration. Prepare 15 ml of collagenase working solution for each heart sample.
- NOTE: Perform all steps on ice unless otherwise indicated.

Method:

1. Euthanize mouse by cervical dislocation.
2. Excise the heart and place it into a 10 cm petri dish containing PBS on ice. Rinse out as much blood as possible. Remove atria and great vessels with fine surgical scissors.
3. Transfer the heart to a fresh 6 cm petri dish and mince the tissue into small pieces (<~1 mm^3) using a scalpel blade. Cover the tissue in a small volume of collagenase solution (~500 µl) to keep them moist during mincing. Note that excessive mincing of the heart tissue may cause considerable cell death
4. Add 5 ml collagenase solution to the dish and transfer the minced tissue into a 70 ml specimen jar using a Pasteur pipette. Mix by gently pipetting up and down to break down the clumps.
5. Incubate the specimen jar at 37 ^{o}C in water bath with shaker for 10 mins, intermittently pipette the tissue gently up and down a few times to break down clumps while avoiding bubble formation.
6. After 10 mins, let the solution settle before transferring the supernatant (suspension cells) to a 50 ml Falcon tube by filtering through a 40 µm sterile cell strainer to remove undigested tissue and debris.
7. Add fresh 5 ml collagenase solution to the remaining undigested tissue in the specimen jar, mix well by pipetting up and down, and repeat steps 5-6 for a further two times. Pool the cell suspension of each heart in same designated 50 ml falcon tube. Centrifuge the cell suspension at 300g for 5 mins, discard the supernatant.
8. Resuspend the pellet in 1 mL of red cell lysis buffer and incubate at RT for 1 min. Note: Warm the red cell lysis buffer to RT prior to use. Centrifuge the tube at 300g for 5 mins and discard the supernatant.
9. Gently resuspend the pellet in 1 ml of ice-cold 2% FBS/PBS and transfer the contents into 1.5 ml Eppendorf tubes. Remove the supernatant carefully by aspiration. Note: handle the tubes gently; try to avoid disturbing the cell pellets at all washing steps which will help to reduce loss of cells.
10. Centrifuge the tube at 300g for 5 mins after washing, discard supernatant and resuspend the pellet in 200 µl of Dead Cell Removal MicroBeads (Miltenyi Biotec), mix well and incubate for 15 min at RT. The beads are conjugated to Annexin V which binds to the dead cell population.
11. During the incubation: prepare 15 ml (per heart) of 1× binding buffer from 20× stock solution; place MACS LS columns in the magnetic field of a QuadroMACS™ Separator and precondition the column with 3 ml of 1× binding buffer. Discard the flow-through.
12. Load cell suspension to the column and slowly add the remaining 12 ml of 1× binding buffer to each column, collect all the flow-through in to a 15 ml tube, placed on ice. The flow-through contains unlabeled live cells.
13. Centrifuge the tubes at 300g for 5 mins. After discarding the supernatant, resuspend the pellet in 1 ml of ice-cold 2% FBS/PBS and transfer the content into a 1.5 ml Eppendorf tube.
14. Centrifuge at 300g for 5 mins, resuspend the pellet in 500 µl of ice-cold 2% PBS/FBS.
15. Transfer 100 µl of cell suspension to a 5 ml FACS tube and dilute with ice-cold 2% PBS/FBS, if necessary. Add DAPI (final: 100 ng/ml) to cell suspension and sort for live single cells ($DAPI^-$) as indicated in Figure 1.
16. When working with *Pdgfra*$^{GFP/+}$ or similar GFP-lineage-tagged mice, you need use unstained cells isolated simultaneously from the heart of a wild type mouse of the same background strain to established gates for GFP^- cells.

References

References

1. Farbehi N, *et al.* (2019) Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury. *eLife* 8:e43882.
2. Chong JJ, *et al.* (2011) Adult cardiac-resident MSC-like stem cells with a proepicardial origin. *Cell Stem Cell* 9(6):527-540.

Related files

Material and method elife paper-farbehi et al_final_bio_protocols2.docx

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Farbehi, N. , Janbandhu, V. , Patrick, R. , Xaymardan, M. , Nordon, R. R. and Harvey, R. P.(2020). Protocols for FACS purification of cardiac total interstitial single cells from adult mouse cardiac ventricles. Bio-protocol Preprint. bio-protocol.org/prep615.
2. Farbehi, N., Patrick, R., Dorison, A., Xaymardan, M., Janbandhu, V., Wystub-Lis, K., Ho, J. W., Nordon, R. E. and Harvey, R. P.(2019). Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury. eLIFE. DOI: 10.7554/eLife.43882